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High Prevalence of Isolates with Reduced Glycopeptide Susceptibility in Persistent or Recurrent Bloodstream Infections Due to Methicillin-Resistant *Staphylococcus aureus*

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Reduced susceptibility to glycopeptides in methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates is considered a risk factor for failure of glycopeptide therapy. We compared the prevalences of MRSA isolates with reduced glycopeptide susceptibility in patients with versus without persistent or recurrent MRSA bloodstream infections. A retrospective cohort study at the University Hospital of Geneva identified 27 patients with persistent or recurrent clonally related MRSA bacteremic episodes over an 8-year period, which included 208 consecutive nosocomial MRSA bacteremic episodes. Vancomycin and teicoplanin MICs were determined by a modified macrodilution assay allowing improved detection of glycopeptide-intermediate MRSA isolates (GISA), characterized by elevated teicoplanin or/and vancomycin MICs ($\geq$4 μg/ml). For 16 patients (59%), their pretherapy and/or posttherapy MRSA isolates showed elevated teicoplanin MICs, among which 10 (37%) concomitantly displayed elevated vancomycin MICs. In contrast, 11 other patients (41%) were persistently or recurrently infected with non-GISA isolates. In comparison, only 39 (22%) of 181 single isolates from patients with no microbiological evidence of persistent or recurrent infections showed elevated teicoplanin MICs, among which 14 (8%) concomitantly displayed elevated vancomycin MICs. Clinical, microbiological, and pharmacokinetic variables for patients persistently or recurrently infected with GISA or non-GISA isolates were similar. Bacteremic patients with a poor response to glycopeptide therapy had a 2.8-fold- and 4.8-fold-higher rates of MRSA isolates displaying elevated teicoplanin and vancomycin MICs, respectively, than patients with single isolates ($P < 0.0001$). Detection of elevated teicoplanin MICs may help to predict a poor response to glycopeptide therapy in MRSA bacteremic patients.

The high worldwide prevalence of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infections is responsible for the intensive use of glycopeptide therapy. A number of clinical studies led to controversial opinions on the efficacy of glycopeptide therapy against severe MRSA infections (8, 35), especially in bacteremic patients (7, 13,18, 24, 27, 40, 49). Failure of glycopeptide therapy against invasive MRSA infections may result from suboptimal vancomycin dosing regimens, yielding inadequate tissue levels at the true sites of serious MRSA infection (8, 35, 40), and/or from its relatively slow bactericidal activity (3, 34, 36).

The emergence of intermediate glycopeptide resistance in some MRSA bacteremic isolates represents an additional concern regarding glycopeptide therapy (7, 8, 18, 24, 28, 35, 49). Most of the MRSA isolates that display low-level, endogenously acquired glycopeptide resistance are hard to detect by phenotypic assays. Vancomycin-intermediate *S. aureus* (VISA) isolates are defined by vancomycin MIC breakpoints of $\geq$4 μg/ml and $<$16 μg/ml and the absence of any vancomycin or teicoplanin resistance determinants (*vanA*, *vanB*, or *vanC*) found in vancomycin-resistant *Enterococcus faecalis* or high-level vancomycin-resistant (vancomycin MIC, 16 μg/ml) *S. aureus* (VRSA) isolates (7, 18, 22, 24, 49). Since VISA isolates are almost uniformly cross-resistant to teicoplanin (28, 44), they are frequently designated glycopeptide-intermediate *S. aureus* (GISA). In contrast to the case for vancomycin, no international consensus has been reached for teicoplanin susceptibility breakpoints in *S. aureus*, which vary from 2 μg/ml according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (10) to 8 μg/ml according to the Clinical and Laboratory Standards Institute (CLSI) (5).

Detection of the GISA phenotype is particularly difficult for isolates displaying heterogeneous resistance to either or both glycopeptides (hGISA isolates), in which only a subset of the microbial population (perhaps as few as 10^{-6} cells) can express glycopeptide resistance (5, 6, 10, 11, 22, 47, 49, 50). hGISA strains are assumed to be precursors of GISA strains, with glycopeptides providing the selective pressure for conversion (11, 24, 31, 33). hGISA detection by using standard microbiological methods is problematic, and this has triggered the development of alternative assays (24, 49), the most popular one being the modified population analysis profile (PAP)-area under the curve (AUC), which utilizes plots of the number of viable colonies against vancomycin concentrations (52, 53). However, PAP-AUC is frequently viewed as labor-intensive and expensive, thus being inappropriate for GISA prevalence studies (18, 24, 33, 49, 50).

Despite the recent adjustment of vancomycin and teicoplanin

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susceptibility breakpoints by CLSI and EUCAST, their predictive value in regard to the outcome of glycopeptide therapy is still debated (16–18, 27, 47, 54). Indeed, several clinical studies reported higher rates of vancomycin treatment failures and increased median times to bacteremic clearance in patients infected with either hVISA or MRSA isolates for which vancomycin MICs (2 μg/ml) were at the upper limit of susceptibility than in those infected with isolates with lower vancomycin MICs ($\leq$1 μg/ml) (8, 16, 18, 21, 27, 29, 35, 46, 47). However, other recent reports challenged the predictive impact of hVISA detection by the PAP-AUC method in regard to the outcome of glycopeptide therapy in MRSA-infected patients (23, 26, 38, 50). Taken together, these mixed results may be explained in part by the lack of sensitivity of the CLSI- and EUCAST-recommended broth microdilution MIC testing method, which leads to underestimation of *S. aureus* glycopeptide MICs (25, 42, 51) as well as underdetection of GISA clinical isolates (51). Therefore, we can speculate that some of the bacteremic isolates scored with vancomycin MICs of 2 μg/ml by microdilution in previous studies were undetected GISA or hGISA, which could potentially contribute to the higher rates of vancomycin failure in those studies (29, 30).

We evaluated the prevalence of decreased glycopeptide susceptibility in patients from a retrospective cohort study at the Geneva University Hospital who presented with microbiologically documented, persistent or recurrent, clonally related MRSA bloodstream infections (BSIs) over an 8-year period. Vancomycin and teicoplanin MICs in consecutive, paired isolates from each patient were evaluated by using a recently described modified macrodilution assay allowing more sensitive detection of GISA isolates (51).

MATERIALS AND METHODS

Study design and data collection. All MRSA isolates from each episode of BSI, validated by at least two separate blood cultures collected at the Geneva University Hospital (48), which is a 2,200-bed tertiary hospital with 41,000 annual admissions, were routinely stored at −80°C before glycopeptide susceptibility testing. This retrospective cohort study identified all patients showing microbiological evidence of persistent or recurrent BSI episodes, recorded from January 1995 to December 2003, via the database of the Central Laboratory of Bacteriology. All MRSA isolates were multidrug resistant and were characterized as hospital acquired (14, 19). A persistent BSI episode was defined as having occurred if a second positive blood isolate was obtained $\geq$72 h after the initiation of glycopeptide therapy. A recurrent BSI episode was defined as having occurred if a second MRSA-positive blood culture occurred at least 2 weeks after completion of glycopeptide therapy. Patients with persistent or recurrent BSI episodes which did not involve clonally related MRSA isolates or who did not receive glycopeptide therapy were excluded. A large number of clinical, microbiological, and pharmacological parameters were extracted from patients' medical and laboratory records: gender, age, types of infection, delay between microbiologically documented recurrent BSI episodes, length of hospitalization, and duration of each BSI episode. The Charlson comorbidity index (4), administration of immunosuppressive drugs, presence of diabetes, infection of central venous catheters or prosthetic/osteosynthetic material, and surgical procedures that occurred within the last 30 days as well as an intensive care unit (ICU) stay of more than 24 h were also recorded.

We also listed previously documented MRSA body colonization, the number of colonized body sites, administration of topical decontamination for active decolonization, and surgery for curative purposes (19). Routine infection procedures did not change substantially during the study period and are described elsewhere (19, 20).

Therapeutic and pharmacokinetic data included antibiotic prescription (vancomycin or teicoplanin, in monotherapy or combined with rifampin) and daily doses and duration of glycopeptide therapy. Median peak and trough plasma levels of vancomycin or teicoplanin, which are routinely assayed by fluorescence polarization immunoassay (FPIA) in our institution, were also included.

Determination of glycopeptide MICs. Each MRSA isolate and two quality control strains were grown in cation-adjusted Muller-Hinton broth (CAMHB) as previously described (51). *S. aureus* strains ATCC 29213 and NRS3 (HIP 5827; provided by the Network of Antimicrobial Resistance in *S. aureus* [NARSA] [www.narsa.net]) were used as glycopeptide-susceptible and GISA quality control strains, respectively (51). Standardized inocula of 10^6 CFU per ml for tube macrodilution MIC and 10^6 CFU per agar plate for agar dilution MIC were prepared from log-phase cultures as described previously (51).

Macrodilution MIC. Vancomycin and teicoplanin MICs were determined by a tube macrodilution assay slightly modified from the M07-A8 (5) and M100-S19 (6) guidelines, as previously described (51). Each antibiotic-containing or control tube was inoculated with ca. 10^6 CFU of each MRSA isolate or quality control strain ATCC 29213 or NRS3 per ml. MIC endpoints were read after 48 h of incubation at 37°C, to improve detection of slow-growing, glycopeptide-"resistant" subpopulations (51).

Modified agar MIC testing method. Each MRSA isolate or quality control strain ATCC 29213 or NRS3 was spread at 10^6 CFU in a 200-μl volume on brain heart infusion (BHI) agar plates supplemented with doubling concentrations (0.5 to 8 μg/ml) of vancomycin or teicoplanin (0.5 to 16 μg/ml) as described previously (51), and growth was evaluated after 48 h of incubation at 37°C. Because a single, undiluted inoculum was uniformly plated onto BHI agar plates, viable counts were scored in a semiquantitative manner as confluent, semiconfluent, or $\leq 10^3$ CFU as described previously (51). The glycopeptide MIC was defined as the lowest antibiotic concentration leading to a $\geq$99.9% reduction in viable counts ($\leq 10^3$ CFU) on BHI agar from the uniformly applied inoculum of 10^6 CFU (51).

Molecular typing. The clonality of consecutive MRSA bloodstream isolates was assessed by a variable-number tandem repeat (VNTR) genotyping method (15). Strain pairs with >85% similarity in the dendrogram were considered clonally related (Bioanalyzer Experiments Clustering software) (15).

When consecutive isolates showed significant increases in glycopeptide MICs during therapy, their clonality was further assessed by multilocus sequence typing (MLST), as previously described (9).

To be scored as GISA, all MRSA isolates had to display consistently a elevated teicoplanin and/or vancomycin MIC ($\geq$4 μg/ml) upon retesting by the modified macrodilution and agar MIC testing methods (51).

Clinical definitions. Patients with persistent or recurrent BSI involving MRSA isolates with teicoplanin and/or vancomycin MICs of $\geq$4 μg/ml, either at the onset or during the course of glycopeptide therapy, were referred to as GISA-infected patients. Conversely, patients with MRSA isolates for which both teicoplanin and vancomycin MICs were $\leq$2 μg/ml in all consecutive isolates from persistent or recurrent BSI were referred to as non-GISA-infected patients.

While most patients were initially treated with glycopeptides for at least 2 weeks, some of them received concomitant administration of rifampin or were switched to other antibiotics for a minimal period of 7 days after completion of glycopeptide therapy.

Statistical analyses. Comparisons between GISA-infected and non-GISA-infected patients in the descriptive analysis were made with the Fisher's exact or chi-square test for categorical variables or with the Wilcoxon rank sum test for continuous variables. Relationships were considered significant when the two-sided *P* value was $\leq$0.05. A univariate analysis of patient demographic and clinical variables was undertaken to identify factors that might have an association with either GISA-related or non-GISA-related BSI. STATA software (9.0; STATA Corp, College Station, TX) was used.

TABLE 1 Characteristics of patients with persistent or recurrent MRSA BSI episodes

Patient no.	Age (yr)	Gender[a]	Duration (days) of glycopeptide therapy: Vancomycin	Duration (days) of glycopeptide therapy: Teicoplanin	Coadministration of rifampin	Days with positive blood culture	Outcome[b]	Interval (days) between first and second isolates	MIC (μg/ml) of Vancomycin: First isolate	MIC (μg/ml) of Vancomycin: Second isolate	MIC (μg/ml) of Teicoplanin: First isolate	MIC (μg/ml) of Teicoplanin: Second isolate	In-hospital mortality
1	75	F	43	0	Yes	5	P	4	2	4	8	8	No
2	87	M	0	8	No	10	P	9	2	4	2	8	Yes
3	58	F	25	0	No	5	P	10	2	4	4	8	Yes
4	2	F	73	26	Yes	27	P	26	4	4	4	8	No
5	68	M	12	3	No	15	R	38	2	4	4	8	No
6	84	M	17	0	No	7	R	50	2	4	4	8	Yes
7	83	M	11	39	No	1	R	126	2	4	2	8	No
8	44	M	101	0	Yes	1	R	137	2	4	2	16	No
9	68	F	16	13	No	3	P	8	2	2	4	4	Yes
10	60	M	33	0	Yes	9	P	8	4	4	4	4	No
11	60	M	39	0	No	2	P	9	2	2	8	8	No
12	65	F	14	0	Yes	11	P	9	2	2	4	4	Yes
13	56	M	5	7	Yes	12	P	11	2	2	4	4	Yes
14	80	F	57	0	Yes	8	R	82	2	2	4	4	No
15	59	M	55	2	Yes	9	R	83	2	2	4	4	No
16	72	F	14	0	Yes	3	R	127	4	4	8	8	No
17	50	M	14	0	No	8	P	7	2	2	2	2	Yes
18	79	M	11	4	Yes	8	P	7	2	2	2	2	Yes
19	86	M	16	0	No	9	P	8	2	2	2	2	Yes
20	53	M	15	0	No	1	P	9	2	2	2	2	Yes
21	72	M	14	9	No	18	P	9	2	2	2	2	Yes
22	58	M	30	0	Yes	1	P	18	2	2	2	2	Yes
23	76	F	62	0	Yes	5	P	20	2	2	2	2	No
24	79	M	24	0	No	1	R	53	2	2	2	2	No
25	93	M	21	13	No	1	R	69	2	2	2	2	No
26	63	M	2	15	No	3	R	82	2	2	2	2	No
27	75	M	14	0	Yes	26	R	118	2	2	2	2	Yes

[a] F, female; M, male.
[b] P, persistent BSI episodes; R, recurrent BSI episodes.

RESULTS

Prevalence of GISA in patients with persistent or recurrent MRSA BSI episodes. Among 208 MRSA bacteremic patients, 181 patients had BSI episodes with single MRSA isolates, in contrast to 27 patients (16%) who showed multiple isolates retrieved from persistent ($n = 16$) or recurrent ($n = 11$) MRSA BSI episodes. Eight patients with persistent or recurrent BSI episodes were not included in the study because they did not have clonally related MRSA isolates or did not receive glycopeptide therapy.

For 16 (59%) of those patients, GISA isolates were detected before, during, or after glycopeptide therapy, as characterized by elevated teicoplanin MICs. Ten of those GISA isolates concomitantly displayed elevated vancomycin MICs. In contrast, 11 other patients (41%) were persistently ($n = 7$) or recurrently ($n = 4$) infected with non-GISA isolates. The main characteristics of the GISA-infected and non-GISA infected patients are presented in Table 1.

Patients with MRSA isolates for which glycopeptide MICs increased during therapy. For 8 patients, glycopeptide MICs for their MRSA isolates significantly increased during therapy. Four patients had persistent MRSA bacteremia, ranging from 5 to 27 days (median, 7.5 days), with a median interval of 9.5 days (range, 4 to 26 days) between the first and second isolates. The median duration of vancomycin therapy for 3 patients was 43 days (range, 25 to 73 days), while the fourth patient was treated with teicoplanin for 8 days followed by a nonglycopeptide antibiotic.

Four patients presented with recurrent bacteremia after an initial BSI episode lasting from 1 to 15 days (median, 4 days), with a median interval of 88 days (range, 38 to 138 days) between the first and second isolates. The median duration of vancomycin therapy was 14.5 days (range, 11 to 101 days), and 2 of those patients were subsequently treated with teicoplanin.

For 7 patients, vancomycin MICs increased from 2 to 4 μg/ml from the first to the second isolate, while it was already elevated (4 μg/ml) in the first isolate of one patient and did not change during therapy. A parallel increase in teicoplanin MIC of the first compared to the second isolate was recorded for 7 patients, namely, from 2 to 8 μg/ml ($n = 2$), 2 to 16 μg/ml ($n = 1$), or 4 to 8 μg/ml ($n = 4$). For one patient, high teicoplanin MICs (8 μg/ml) were recorded in both pretherapy and subsequent isolates.

The clonal relationships of subsequent isolates for which teicoplanin and/or vancomycin MICs increased during therapy were confirmed by MLST (see below).

Patients with MRSA isolates displaying elevated glycopeptide MICs throughout therapy. For 8 patients, teicoplanin MICs of 4 μg/ml ($n = 6$) or 8 μg/ml ($n = 2$) were already recorded in pretherapy isolates and did not change during therapy. For one patient, both pretherapy and consecutive MRSA isolates concomitantly displayed elevated vancomycin and teicoplanin MICs (4 μg/ml). Five patients had persistent MRSA bacteremia, ranging from 3 to 12 days (median, 9 days), and a median interval of 9 days (range, 8 to 11 days) between the first and second isolates. The

TABLE 2 Prevalences of GISA and non-GISA isolates in patients with nonrecurrent versus recurrent/persistent MRSA BSI episodes

Glycopeptide MIC[a] (μg/ml)	No. (%) of patients with MRSA BSI episodes: Nonpersistent/nonrecurrent ($n = 181$)	Persistent/recurrent ($n = 27$)	P
≤2	142 (78)	11 (41)[b]	<0.0001
≥4			
Teicoplanin	39 (22)	16 (59)[c]	<0.0001
Vancomycin[d]	14 (8)	10 (37)[e]	<0.0001

[a] MICs were scored by macrodilution assay read at 48 h.
[b] All subsequent isolates from each patient were consistently susceptible to teicoplanin and vancomycin.
[c] At least one of the paired isolates from each patient displayed an elevated teicoplanin MIC.
[d] All isolates with elevated vancomycin MICs also displayed elevated teicoplanin MICs.
[e] At least one of the paired isolates from each patient displayed elevated vancomycin and teicoplanin MICs.

median duration of vancomycin therapy was 16 days (range, 5 to 39 days), and 2 of those patients received subsequent courses of teicoplanin therapy.

Three other patients presented with recurrent bacteremia after an initial BSI episode lasting from 3 to 9 days (median, 8.5 days), and the median interval between the first and second isolates was 83 days (range, 82 to 127 days). The median duration of vancomycin therapy for these patients was 55 days (range, 14 to 57 days).

Patients infected with non-GISA isolates throughout therapy. Seven patients presented with persistent non-GISA BSI episodes ranging from 1 to 18 days (median, 8 days), and the median interval between the first and second isolates was 9 days (range, 7 to 20 days). Two patients were listed as having a brief (1-day) initial BSI episode, followed by a relapse with the same, clonally related isolate at 9 and 14 days, respectively, during the course of glycopeptide therapy. The median duration of vancomycin therapy was 15 days (range, 11 to 62 days) for these patients, and two patients were subsequently treated with teicoplanin therapy.

Four patients presented with recurrent non-GISA bacteremia after an initial BSI episode lasting from 1 to 26 days (median, 2 days), and the median interval between the first and second isolates was 75.5 days (range, 53 to 118 days). The median duration of vancomycin therapy was 17.5 days (range, 2 to 24 days) for these patients, and two patients received additional courses of teicoplanin therapy.

Prevalence of GISA and response to glycopeptide therapy. In the group of 182 patients with single isolates showing no microbiological evidence of persistent or recurrent infections, only 39 isolates (22%) showed elevated teicoplanin MICs, among which 14 (5%) also displayed elevated vancomycin MICs. Compared to the group of 182 patients with single isolates, the prevalence of GISA bacteremic isolates was significantly higher ($P < 0.0001$) in the group of 27 patients with persistent or recurrent BSI episodes (Table 2). Indeed, bacteremic patients with a poor response to glycopeptide therapy had 2.8-fold- and 4.8-fold-higher rates of MRSA isolates displaying elevated teicoplanin and vancomycin MICs, respectively, than patients with single isolates ($P < 0.0001$).

Clinical and laboratory parameters for GISA-infected versus non-GISA-infected bacteremic patients. Twenty-nine clinical, microbiological, and pharmacological parameters for patients with persistent or recurrent GISA versus non-GISA BSI episodes were compared (see Table S1 in the supplemental material). To increase the power of the statistical analysis, data from patients with persistent and recurrent BSI episodes involving GISA isolates detected before, during, or after glycopeptide therapy were pooled into a single group and compared to those from patients infected with non-GISA isolates. Demographics, underlying conditions, and therapeutic modalities for GISA-infected and non-GISA-infected patients were not significantly different, except for the MRSA colonization rate, which was significantly higher ($P < 0.05$) in GISA-infected (100%) compared to non-GISA-infected (55%) patients. These data were essentially confirmed by univariate analysis that showed that no other factor besides prior colonization by MRSA was associated with either GISA-related or non-GISA-related BSI episodes (see Table S2 in the supplemental material). Neither the coadministration of rifampin nor subsequent teicoplanin therapy seemed to influence occurrence of GISA versus non-GISA BSI episodes.

Finally, there was a trend, which did not reach significance, toward a higher overall in-hospital mortality for patients infected with non-GISA ($n = 7$) compared to GISA ($n = 6$) isolates (64% and 38%, respectively; $P = 0.252$). Further analysis indicated a significantly ($P = 0.018$) higher overall mortality for patients with persistent ($n = 11$) compared to recurrent ($n = 2$) BSI episodes. However, only 2 patients died within 30 days after the onset of BSI. Among those 13 patients, only 3 had endocarditis, namely, 2 GISA-infected patients and one non-GISA-infected patient.

Epidemiology of nosocomial MRSA isolates during the study period. A dendrogram of all paired isolates from the 27 patients with persistent or recurrent MRSA BSI episodes, whose clonal relationships were analyzed by the VNTR genotyping method (15), is shown in Fig. S1 in the supplemental material. In line with the notion that nosocomial infections worldwide are due to a few hospital-acquired MRSA clones (39), the 27 paired isolates were clustered in two distinct groups of hospital-acquired MRSA clones showing <50% similarity. The first group includes 19 strain pairs isolated from 1998 to 2003, which corresponds to the acquisition of the South German clone (ST228) that became predominant after 1999 (14). Five isolates of this VNTR cluster displayed ST269, which is closely related to ST228. The second group includes 8 paired isolates of hospital-acquired MRSA clones that were present before introduction of the South German MRSA clone (2), two of them being characterized by ST30 and ST45. Interestingly, glycopeptide MICs increased in 3 different ST types (ST30, -45, and -269) of MRSA isolates during BSI therapy (see Fig. S1 in the supplemental material).

DISCUSSION

Despite numerous studies reported during the past 14 years (summarized in references 24 and 49), the clinical significance of GISA infections remains controversial. This situation is mainly due to technical problems in assessing the GISA phenotypes of MRSA isolates and in recruiting adequate numbers of GISA- and hGISA-infected bacteremic patients for comparative studies. In contrast to studies performed before 2009, which were mostly descriptive and/or included low numbers of VISA or hVISA patients (49), five recent reports provided more exhaustive comparisons of the clinical features and treatment outcomes for hVISA versus non-VISA patients (1, 23, 26, 31, 38). While none of those studies demonstrated any increase in mortality rates of hVISA compared to non-hVISA patients, more contrasting results were reported for other treatment outcomes. Whereas two studies reported a prolonged bacteremia duration and greater rates of complications for hVISA compared to non-hVISA patients (1, 31), three other reports revealed no significant association between the presence of hVISA and persistent bacteremia (26, 38, 50). Furthermore, a prospective study showed lower rates of infections, including bacteremia, and no influence on treatment outcome for hVISA-infected compared to non-hVISA-infected patients, as determined by PAP-AUC criteria (23).

As mentioned above, technical problems associated with detection of GISA, in particular hVISA, isolates may have played a significant role in the conflicting treatment outcome data reported in the aforementioned clinical studies (1, 23, 32, 38). The phenotypic expression of low-level glycopeptide resistance is variable, being significantly influenced by several technical parameters, including the compositions of liquid or solid test media and various time frames and inoculum sizes (24, 28, 49, 51). Since broth microdilution and agar dilution assays use inadequate inocula and too-short incubation periods for reliable detection of some hGISA isolates (25, 42, 47, 51), alternative GISA detection methods, such as the PAP-AUC, Etest macromethod, and Etest GRD, have been proposed (summarized in references 24 and 49) and repeatedly tested *in vitro* (26, 41, 45, 50, 55). Despite their merits, these alternative GISA detection methods have generated complex results that can hardly be integrated into the current schemes of glycopeptide MIC breakpoints. How each of these alternative GISA detection assays, e.g., the PAP-AUC versus Etest macromethod, contributed to the conflicting treatment outcomes for hVISA- versus non-hVISA-infected patients observed in previous studies (1, 23, 26, 32, 38, 50) has not been elucidated.

To improve the detection of hGISA or GISA by standard MIC criteria, we developed and validated a slightly modified macrodilution MIC assay method, which uses higher inocula than the broth microdilution MIC method and extends the incubation period to 48 h at 37°C to optimize the detection of slowly growing resistant subpopulations (51). The increased sensitivities of the macrodilution and agar testing methods over the microdilution assay markedly improved the rates of detection of GISA isolates, as defined by teicoplanin and vancomycin MIC values of $\geq$4 μg/ml, by ca. 10-fold and 4-fold, respectively (51). We also noticed that the day-to-day reproducibility of intermediate teicoplanin resistance expression was more regular than that of intermediate vancomycin resistance (51). Furthermore, a strict distinction between hVISA and VISA was found to be unnecessary, because several isolates could switch from the hVISA to VISA phenotype or vice versa in experiments done on different days (51). Altogether, detection of elevated teicoplanin MICs was a reliable marker of the GISA phenotype and facilitated the detection of isolates expressing intermediate vancomycin resistance (44, 51). In agreement with previously documented rates of teicoplanin-to-vancomycin cross-resistance of approximately 40% of MRSA bloodstream isolates (51), we found that 63% versus 36% of teicoplanin-intermediate isolates were cross-resistant to vancomycin in bacteremic patients with persistent or recurrent MRSA BSI episodes versus those with single isolates, respectively.

While emergence of endogenous vancomycin and teicoplanin resistance is a stepwise process involving several mutations in key regulatory genes (24, 43), the molecular differences between isolates displaying combined low-level resistance to both teicoplanin and vancomycin and those resistant to teicoplanin alone has not been elucidated.

A majority of persistent or recurrent MRSA BSI episodes observed from 1998 to 2003 were due to the South German clone ST228 or relatives, which became predominant in our institution after 1999 (14). This MRSA clone shift was also documented by pulsed-field gel electrophoresis data of MRSA bacteremic isolates from 1995 to 2001 (2). ST228 is an SCC*mec* type 1 and *agr* type 2 MRSA clone that was also highly predominant in orthopedic device-related MRSA infections occurring in our institution from 2000 to 2008 (12). It is remarkable that *in vivo* emergence of GISA during therapy of persistent or recurrent MRSA BSI episodes occurred not only in ST228-derived isolates but also in two ST30 and ST45 isolates retrieved in 1995 and 1997. Thus, the influence of the clinical background on *in vivo* emergence of GISA may be less important than previously suggested (37).

This study has some limitations. This was a single-center, retrospective study, and the clinical and epidemiological management of BSI episodes likely evolved during the 8-year period. Failure of glycopeptide therapy in MRSA bacteremic patients is clearly multifactorial, as found in several studies, being influenced by several demographic and clinical risk factors in addition to emergence of reduced susceptibility to glycopeptides. The difficulty in recruiting adequate numbers of GISA- and hGISA-infected bacteremic patients for comparative studies leads to small sample sizes that prevent detailed analysis of risk factors. In our study, patients with persistent and recurrent GISA BSI episodes had to be analyzed collectively in view of the small numbers.

In conclusion, the development of simple, more sensitive MIC assays for detecting GISA isolates should prompt the design of a multicenter, prospective study for evaluating the clinical impact of the GISA phenotype and other risk factors on the outcome of glycopeptide therapy in MRSA bacteremic patients. This approach should also facilitate the detection of more specific molecular markers of endogenous low-level resistance to vancomycin versus teicoplanin.

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